MUTAGENIC EFFICACIES OF TRIACONTANOL IN *CHARA BRAUNII* Gm. (CHAROPHYTA) WITH REFERENCE TO ITS APPLICATION IN CHROMOSOME ANALYSIS

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ABSTRACT. – 1-Triacontanol, a saturated primary alcohol, has been investigated for its mutagenic and radiometric activities on *Chara braunii* (n = 14) for the first time. The nuclear and chromosomal aberrations like micronuclei, trinucleate cells, chromosome forward, scattered metaphase, chromatid separation, laggard chromosomes, spindle shifting, chromosome condensation, chromosome clumping, chromosome clearity and chromosome groups at metaphase were recorded. The application of triacontanol in chromosome analysis has been suggested.

RÉSUMÉ. – Le triacontanol, alcool primaire saturé, a été expérimenté en raison de ses actions mutagène et radiométrique sur *Chara braunii* (n = 14) pour la première fois. Des anomalies nucléaires et chromosomiques ont été observées, telles que micronoyaux, cellules trinucléées, chromosome «trainard», métaphase dispersée, chromatides séparées, fuseau anormal, chromosomes raccourcis ou agglutinés, chromosomes par groupes à la métaphase, etc. Les effets du triacontanol pourraient être utilisés dans l'analyse chromosomique.

KEY WORDS : Charophyta, chromosomal aberrations induced by 1-Triacontanol.

INTRODUCTION

1-Triacontanol, a saturated primary alcohol, is represented by the molecular formula $CH_3\ (CH_2)_{28}\ CH_2\ OH$ and its molecular weight is 438. 80. It was isolated from a phanerogam, *Medicago sativa* L. (alfalfa or lucern) of family Fabaceae.

The present available literature on algae (ABBAS, 1963; BHATNAGAR, 1981; CHATTERJEE and SHARMA, 1972; DELAY and CARPENTIER, 1955; DODGE, 1964; MOUTSCHEN, DAHMEN and GILLET, 1956; NOOR, 1966; SARMA, 1957, 1960, 1962; SARMA and CHAUDHURY, 1976; SARMA and TRIPATHI, 1973, 1974 a, b, 1976 a, b; SHYAM and SARMA, 1976; SINHA,

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TABLE I

Name of the chemical : Triacontanol (TRICA)
Molecular formula : $CH_3(CH_2)_{28}CH_2OH$
Molecular Weight : 438. 80
Solvent : Distilled water
Concentrations used : Control, 0.25 ppm, 0.50 ppm, 0.75 ppm, 1.00 ppm.
Experimentation period : 1.00 hr, 2.00 hrs, 3.00 hrs, 4.00 hrs.
Experimental plant : *Chara braunii* Gm.
Chromosome number : n = 14
Chromosomal aberrations :

Concentrations															
Control		1 ppm				0.75 ppm				0.50 ppm				0.25 ppm	
Duration of the treatment (in hrs)															
1 2 3 4		1 2 3 4				1	2	3	4	1	2	3	4	1	2	3	4
ABERRATIONS OBSERVED															
						–	–	–	–	–	en	–	–	–	–	–	cd
N		L				mn	md	–	L	–	mn	–	–	–	–	–	–
O		E				–	–	–	–	–	–	–	L	–	–	–	–
		T				–	–	–	–	–	–	–	du	–	–	–	–
A		H				–	–	–	–	–	tn	–	–	–	–	–	–
B		A				–	–	–	–	–	cf	–	–	–	–	–	cf
E		L				–	–	–	–	–	sm	–	–	–	–	–	sm
R						–	–	–	–	–	cs	–	–	–	–	–	cs
R		E				–	–	–	–	–	–	lag	–	–	lag	–	lag
A		F				–	–	–	–	–	–	–	–	–	–	–	cc
T		F				–	–	–	–	–	–	–	–	–	–	–	clp
I		E				–	–	–	–	–	–	–	–	–	–	–	cg
O		C				–	–	–	–	–	–	–	mn	–	–	–	cla
N		T				cb	–	–	–	–	–	–	–	–	–	–	–
S		S				–	–	–	–	–	–	–	–	–	–	–	–
						–	–	–	–	–	–	–	–	–	–	–	–

Abbreviations :

en : enlarged nucleus
mn : micronuclei
md : mitotic delay
L : lethal effects
du : dumb-bell shaped cells
tn : trinucleate cells
cf : chromosome forward
sm : scattered metaphase
cs : chromatid separation
lag : laggard chromosome
cc : chromosome condensation
clp : chromosome clumping
cg : chromosome groups
cla : chromosome clearity

1960; SINHA and AKHAURAY, 1970; SINHA and SINHA, 1971; TURNER, 1970) indicates clearly that there is absolutely no work on effect of triacontanol on any of the algal groups. The present investigations are therefore pioneer for group of macrophytes.

MATERIAL AND METHODS

Triacontanol was procured from Sigma Laboratories (U. K.) and the living specimens of *Chara braunii* were collected from Balapur village in Bareilly district of U. P. India, during December - January 1984. The plants were maintained in soil - water culture medium prepared by sterilized soil and water of the same pond. Various concentrations of triacontanol were prepared in distilled water. Young growing plants were transferred to these fractions for varying periods followed by the thorough washing in distilled water and subsequent fixation in 1:2 acetic - alcohol (Carnoy's fluid). After 24 hours, the fixed fertile tips were transferred to 70 % alcohol. GODWARD's (1948) iron - alum aceto-carmine method and feulgen were used separately for smearing. Microphotographs were taken for the nuclear abnormalities from temporary preparations which were made permanent subsequently by using tertiary butyl alcohol and euparal schedule. Six plants were fixed without treatment at regular intervals for comparison along with the fixation of six treated plants in each replicate.

OBSERVATIONS AND DISCUSSIONS

As revealed by Table I, the mitotic cycle of *Chara braunii* (n = 14) was influenced notably at various stages and remarkable genomic mutations have been recorded. A comprehensive study of these effects is described below.

A. Interphase :

During interphase, no visible mutations could be observed but in 0.5 and 0.25 ppm (parts per million) for 2 and 4 hours respectively, the interphase nuclei were enlarged having prominent dot like chromatin along with two darkly stained bodies in close vicinity with the nucleous in almost all the cells. Concentration beyond 0.5 ppm showed lethal effects.

B. Prophase :

With the onset of mitotic division, triacontanol enters into the cell cycle of antheridial filaments and detain the divisional stages resulting into the mitotic delay (0.75 ppm for 2 hrs and beyond). Formation of micronuclei by the fragmentation of chromatin network into smaller units was quite frequent in 0.5 ppm for 2 hrs. The frequency of micronuclei increased subsequently with an increase in concentration and the duration of treatment upto 0.75 ppm for 1 hr (very rarely 2 hrs). The treatment of 0.75 ppm for 4 hrs and more yielded dumb-bell shaped cells.

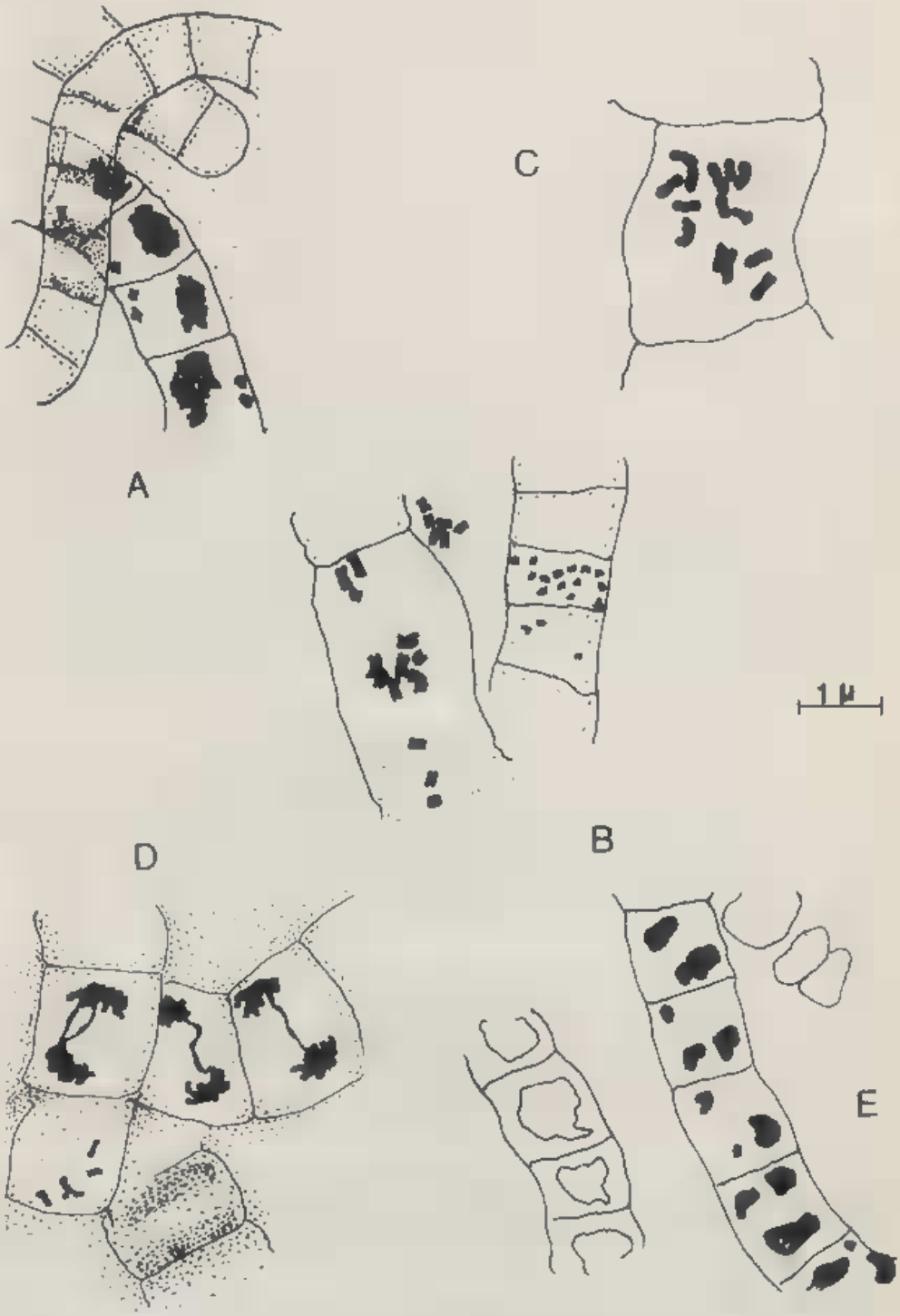

Plate 1. – *Chara braunii* Gm. A : chromosome forward. B : chromatid separation. C : scattered metaphase. D : chromatin bridge. E : trinucleate cells.

C. Metaphase :

During this stage of cell division, maximum aberrations were recorded perhaps due to the appreciably large and prominent chromosomes, their attachment with the spindle fibres, etc. The occurrence of chromosome forward (cf), scattered metaphase (sm), chromatid separation (cs), chromosome condensation (cc) and clearity of chromosomes during metaphase indicate clearly the influence of triacontanol on the spindle fibres and their morphogenesis. Almost all the metaphasic aberrations were found in the treatments of 0.25 and 0.5 ppm for 4 hrs and 2 hrs respectively. The treatment of 0.5 ppm beyond 3 hrs only shows some micronuclei and some dumb-bell shaped cells. The optimum aberrations were in 0.25 ppm for 4 hrs. Condensation of chromosomes and chromatid separation are the effects which may help appreciably in karyotypic investigations and chromosome analysis (Pl. 1, A - B - C).

D. Anaphase :

The movement of chromosomes towards different poles under the influence of spindle metabolism seems to be influenced much by triacontanol. Chromosome breakage, though not observed quite distinctly during metaphase, leaves some acentric chromosomes. During polar movement of chromosomes, such chromosomes in absence of a centromere do not move towards any of the poles and remain as such at the equatorial plate. The frequency of these laggards was maximum in 0.25 ppm for 2.00 hrs and 2.30 hrs which constantly deteriorated with an increase in the period of treatment ultimately producing lethal results. A few cells having laggards were also seen in 0.5 ppm for 3 hrs. Splitting of spindles during anaphase results into the formation of four groups of the chromosomes. Chromatin bridges during anaphase were rarely seen in 1hr treatment of 0.75 ppm (Pl. 1, D-E).

E. Telophase :

Complete mitosis upto the stage of telomere formation and the formation of spermatozoids was not seen perhaps due to lethality of triacontanol during long treatments. Many cells were deformed and did not retain viability. The living plants in 0.25 ppm (after 5 hrs), in 0.5 ppm (after 4.30 hrs), in 0.75 ppm (after 2 hrs) and in 1 ppm (after 1.30 hr) were not looking healthy and were at the verge of disintegration.

An overall observation of triacontanol activity during mitotic cycle in *Chara braunii* shows an increase in the frequency of chromosomal aberrations with an increase in concentration and duration of treatment constantly upto point «M» (Pl. 2). A steep downfall in the frequency can be seen between 0.25 ppm and 0.5 ppm.

The system of classification propounded by WOOD and IMAHORI (1965) for world Charophytes is based entirely on gross morphological features which is not true in many cases on karyological grounds as discussed in sufficient detail by various Charologists (cf. BHATNAGAR, 1981, 1983). The karyotypic

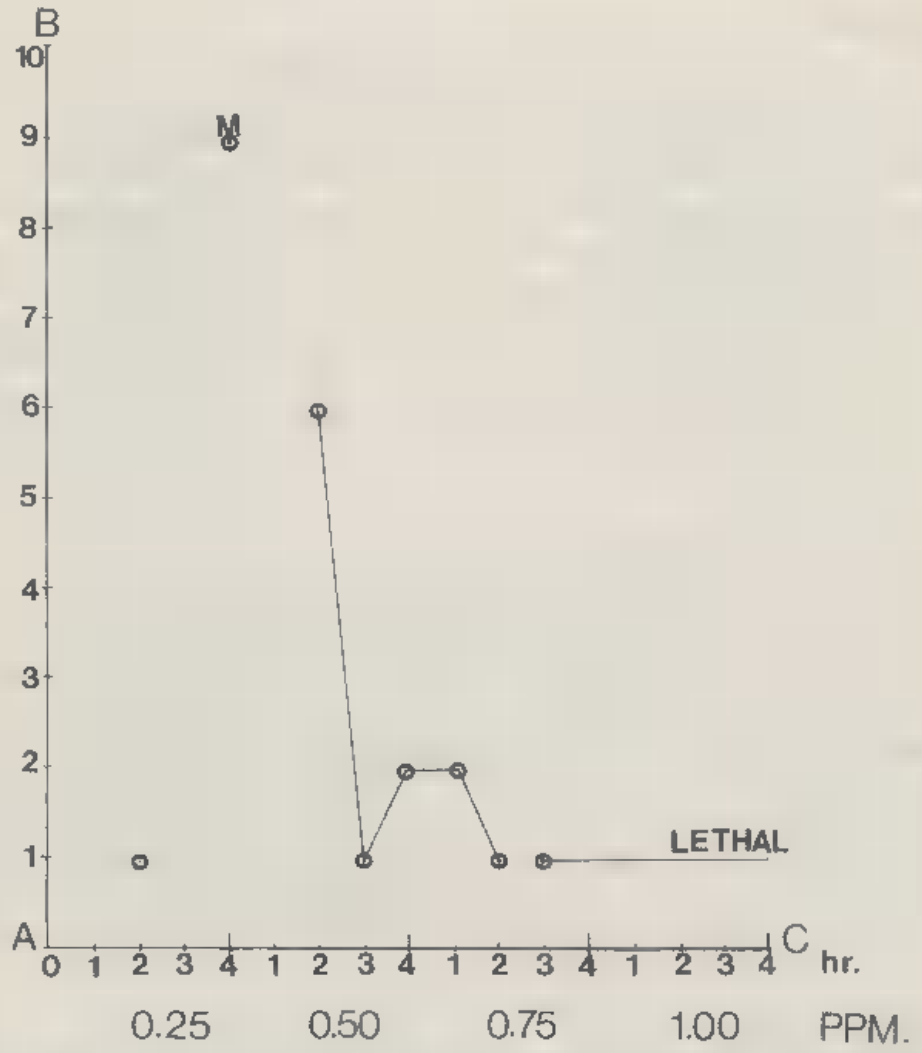

Plate 2. – AB : types of aberrations. AC : concentrations (in ppm) and duration (in hr).

investigations in numerous Charophytes genera also showed a high degree of variability within the same forma. The authors are of the view that these disparities in the karyotype were due to the lack of a definite method for locating centromeric position. In the past years, it could be done merely from the anaphasic configuration and by the shape of chromosomes at the poles. We also admit the practical problems with the previous workers in deciding the exact position of centromere in algal chromosomes owing to a high degree of conspecificity of chromosomes in this group.

The degree of chromosome susceptibility towards various radiomimetic substances, mutagens and radiations can help a lot in deciding the evolutionary sequence and speciation within this group like numerical mutations (polyploidy).

The polyploid races are treated as most evolved and in the same way, the response of Charophytic chromosomes towards inducing agents may help in differentiating the evolved and primitive forms of a species.

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